Urine based newborn screening study applying high-resolution NMR spectroscopy in Turkey

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Background: Approximately 1:1000 neonates are affected by congenital metabolic diseases in central Europe and 1:500 in Turkey. Undetected and untreated these diseases can lead to irreversible organ failures, invalidity or death. Fully automated NMR spectroscopy of body fluids is used as an analytical approach for diagnosis of known, but also as yet unknown inborn errors of metabolism.

Body fluid ^{1}H-NMR spectroscopy: NMR spectroscopy of body fluids can be a complementary technique to find the diagnosis of metabolic diseases. ^{1}H-NMR spectroscopy of body fluids shows the majority of proton-containing compounds and therefore provides an overall view of metabolism. NMR spectroscopy of body fluids may be considered as an alternative analytical approach for diagnosing known, but also as yet unknown, inborn errors of metabolism.

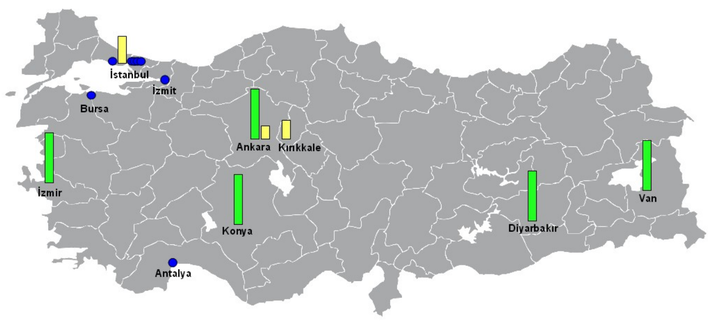

Figure 1: Presentation of the current status of the ongoing study. Green bars indicate active centers that have completed sample collection, yellow bars centers in process of collection, blue prospective centers which have not started yet.

Statistical analysis: The statistical analysis and quantification of metabolite concentrations will be based on a combination of 1D-spectra and fast 2D-J-resolved spectra. Here, the 2D-spectra support the secure identification and deconvolution based quantification of metabolites identifying line position and multiplet structure as obtained from a J-resolved 2D-spectrum. Further statistical modules developed at Bruker BioSpin GmbH use a normal model for untargeted screening and allow the detection of unknown diseases for Turkish babies. In addition, targeted screening is used to detect known diseases.

Study design: Open, one-arm, non-interventional study. Data from 1000 healthy Turkish neonates will be included to explore the range of variation (concentration and chemical shift) of specific metabolites in this population and to identify the pathological thresholds of these metabolites in urine.

Study Objectives: Primary objective of the study was to explore the range of variation (concentration and chemical shifts) of specific metabolites without clinically relevant findings. Secondary objective was the integration of the results from a healthy population of neonates into an NMR-knowledge base to perform routine and completely automatic screening for congenital metabolic diseases using targeted and untargeted approaches out of one measurement per sample.

Patients and methods: Urine samples of 690 neonates from 8 centers in Turkey were investigated by using fully automated NMR spectrometers in two different laboratories (INFAI and Bruker).

Results: It is known that healthy neonates can have different pathological metabolites in high concentration after birth. It is important to compare concentration ranges of these pathological metabolites with clinical data to reach a reliable conclusion for the future development of the child.

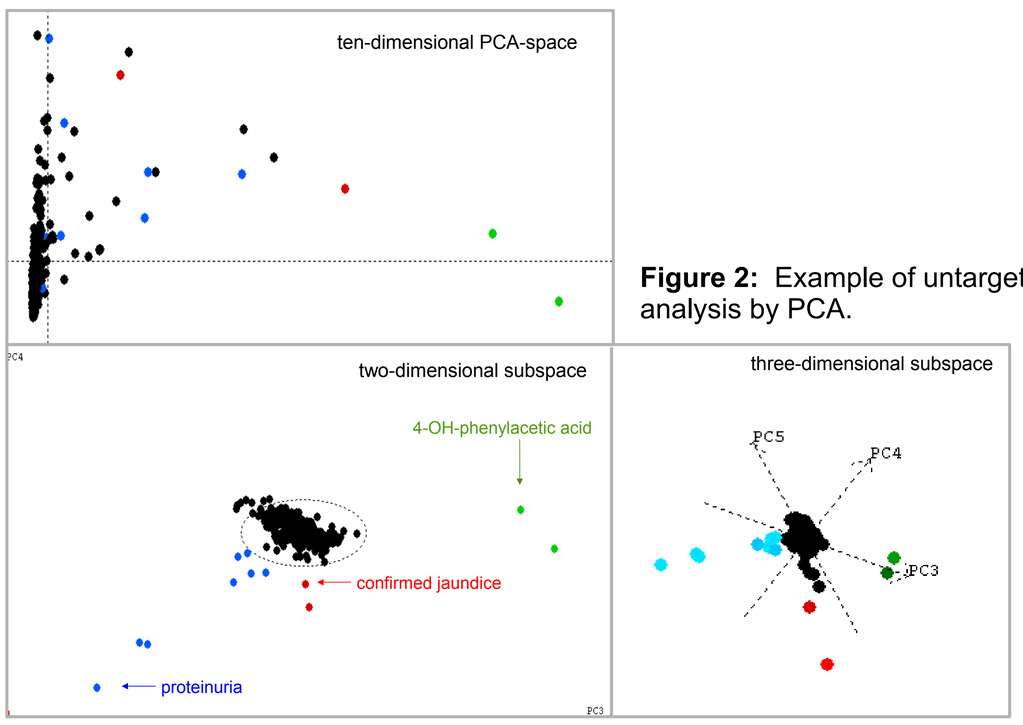

Figure 2: Example of untargeted analysis by PCA.

Statistical analysis of spectra was used to identify suspicious samples. Figure 2 gives an example of this type of analysis. Principal component analysis (PCA) generates a representation where each sample is shown as an individual point. Suspicious samples become evident as outlier points. In the example, three types of outlier samples are observed, shown in different colors. Spectral analysis shows high concentration of a fingerprint metabolite. Two of the subjects were confirmed to have suffered from jaundice, one had to be treated in an intensive care unit. For ten subjects high concentrations of macromolecules have been observed in the aliphatic region. An external laboratory identified albumin, which indicates manifest nephropathy. Several subjects were observed with 4-hydroxyphenylacetic acid [1] in high concentration, but no unusual findings were reported in clinical data.

Quantification: After identification of metabolites in NMR spectra, simple integration of selected metabolites of interest yields fully quantitative information on metabolite concentration. Figure 3 gives an example. The concentrations of D-galactose (246 mmol/mol creatinine) and 4-hydroxy phenylacetic acid (1274 mmol/mol), double of what has been described as pathological (631 mmol/mol) [4].

Follow-up investigation: It is necessary to monitor children which were identified by statistical analysis. We plan to collect urine samples from these subjects at a later point in time. This will allow to distinguish cases were metabolite levels have returned to normal from pathological cases, thus significantly reducing the number of false positive screening results.

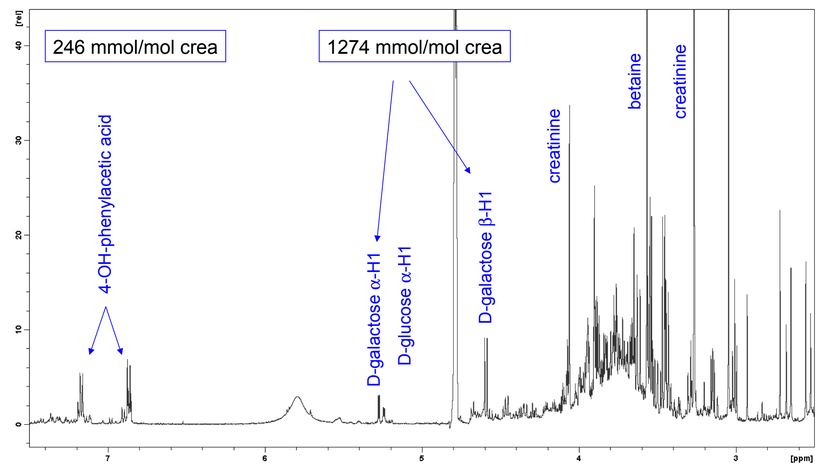

Figure 3: Quantification demonstrated on a subject where both 4-OH-phenylacetic acid and D-galactose are found in pathological concentration[4].

Fully automatic quantification: Further metabolites were observed in high concentration: 3-hydroxybutyric acid [2] (child had symptoms of ketosis), myoinositol (found associated with aminoaciduria [3]), and D-galactose [4] were detected. Concentrations were determined fully automatically for all 690 samples; a histogram of the distribution of concentrations is given in Figure 4.

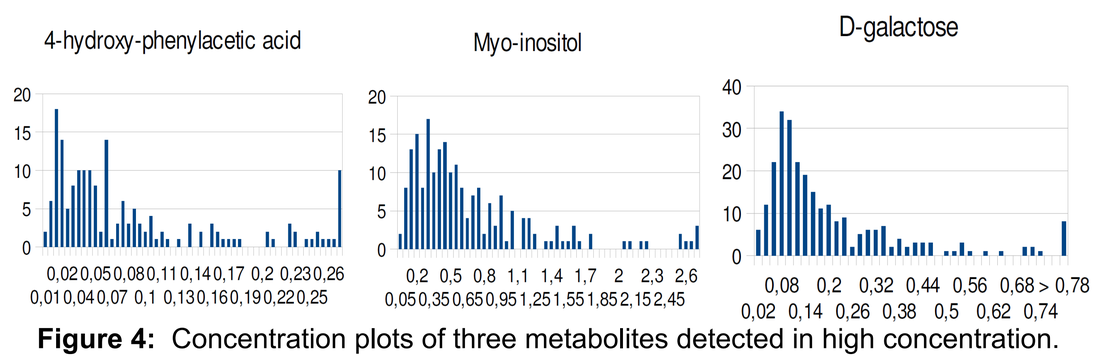

Figure 4: Concentration plots of three metabolites detected in high concentration.

Reproducibility between laboratories: All samples were measured in duplicate, in INFAI's lab in Cologne and in Bruker's lab in Karlsruhe. In Figure 5, the results of a PCA analysis applied on the combined data set (both laboratories, Lab-1: yellow, Lab-2: blue) are shown. Each pair of samples is represented by a pair of markers connected by a blue line. The small insert plot shows a zoom into the black rectangle. It is found that the two aliquots of each initial sample measured independently in the two labs are always represented by points which are in close proximity demonstrating excellent reproducibility.

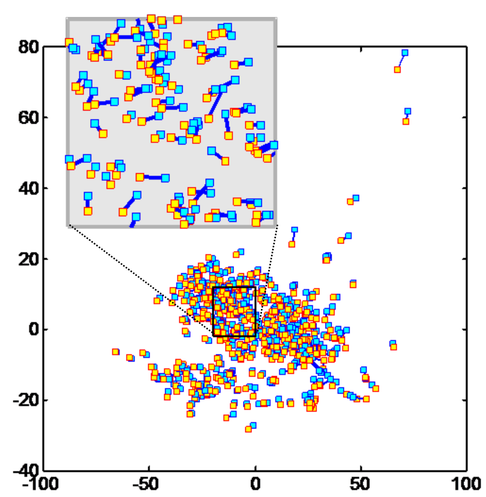

Figure 5: PCA analysis comparison between two laboratories (Lab-1: Bruker, Lab-2 INFAI)

Conclusion:

- Establish a statistical normal model of 'healthy' urine spectra
- Establish automatic quantification and 'normal ranges' for common metabolites
- Identify pathological metabolites that can be used as disease markers
- Integrate into a comprehensive screening test for newborn health
- The statistical analysis and quantification of metabolites allow developing a normal model in a specific population and also a general assessment of neonate health state

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References

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